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Trends in *Neisseria meningitidis* Serogroups Amongst Suspected Cerebrospinal Meningitis Patients in the Meningitis Belt of Ghana- A Five-Year Retrospective Study

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Trends in *Neisseria meningitidis* Serogroups Amongst Suspected Cerebrospinal Meningitis Patients in the Meningitis Belt of Ghana- A Five-Year Retrospective Study

Directed by: Professor Jun Yong Choi

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Submitted to the Department of Global Health Security

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in partial fulfilment of the requirement for the degree of

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Azure Stebleson

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This certifies that the Master's Thesis
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Dedication

I dedicate this august work to my loving parents, Mr. Abaane Agambire and Mrs. Mary Adongo, as well as my ever-caring sister, Courtney Awehor-Niah Gambilla, for their unflinching support throughout my educational journey. It is also dedicated to Mr. John K. Apaalah (formerly of the GCB Bank Limited, Ghana) and my late uncle, Mr. John Ayuni Gambilla. Their collective efforts and contributions towards bringing me this far have been invaluable.

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Abbreviations

PCR	Polymerase Chain Reaction
CSM	Cerebrospinal Meningitis
WHO	World Health Organization
UK	United Kingdom
MACV	Meningococcal A Conjugate Vaccine
CSF	Cerebrospinal Fluid
IMD	Invasive Meningococcal Disease
CLSI	Clinical and Laboratory Standards Institute
CFR	Case-fatality Ratio

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Abstract

Background: *Neisseria meningitidis* is classified based on its surface polysaccharide capsule's antigenicity into thirteen (13) serogroups. More than 90% of the globally invasive meningococcal infections were caused by serogroups, A, B and C. Serogroup A was the major cause of meningococcal meningitis epidemics in the African meningitis belt prior 2010 when a monovalent meningococcal A conjugate vaccine (MenAfriVac) was introduced in the region.

Purpose: This paper aimed to examine a five-year trend of *Neisseria meningitidis* serogroups in the meningitis belt of Ghana.

Methodology: PCR confirmed laboratory results of all suspected cases of cerebrospinal meningitis (CSM) from 2016 to 2020 were obtained from the Tamale Public Health Laboratory and the data were subjected to trend analysis using SPSS

Results: Out of the 395 confirmed *Neisseria meningitidis* cases, 71.4% and 24.6% were serogroups Wand X, respectively. Serogroups; B, C and the NG accounted for 0.5%, 0.5% and 3%, respectively. There is a significant upward trend of serogroup X (p=0.01) and downward trend of the serogroup W (p=0.01) and serogroup C (p=0.05). The serogroups B and C are also showing a downward trend, and the NG serogroups are in the upward trajectory. However, the serogroups; B, and NG yearly are insignificant (with p-values; 0.78, and 0.21, respectively).

Conclusion: There has been the emergence of serogroup X, a non-vaccine type *Neisseria meningitidis* serogroup, as the predominant *Neisseria meningitidis* serogroup, and is on the upward trajectory since 2016 in the wake of a declining serogroup W after the introduction of the meningococcal polysaccharide ACW vaccine following the outbreak of serogroup W in the Upper West region in 2016.

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Keywords: *Neisseria meningitidis*, Serogroups, Real-Time Polymerase Chain Reaction (rt-PCR), Meningitis Belt, Meningococcal, Tamale Public Health Laboratory, Cerebrospinal meningitis (CSM).

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CHAPTER ONE
1.0 INTRODUCTION

1.1 Background

Bacterial meningitis is a severe acute infection of the fluid surrounding the brain and the spinal cord resulting in the inflammation of the meninges that can rapidly result in death. Bacterial meningitis can be fatal in about 50% of all cases if treatment is not given. Even when early diagnosis is made and adequately treated, about 8-15% of cases result in death, usually within 24 and 48 hours after the onset of symptoms[1]. In Africa, 25% of individuals infected could experience neurologic sequelae, including brain damage, hearing loss, and learning disabilities despite the treatment with the recommended antibiotics[2]. According to the WHO, meningitis remains a universal public health challenge in countries worldwide where cases and outbreaks are highly dreaded, with the global number of deaths, as a result, estimated to be 380, 000 annually. The highest outbreaks and deaths as a result of meningitis occur in the developing countries[3]. In the year 2009, an estimated number of 80,000 suspected cases with 4000 resulting in death were reported in of Africa's meningitis belt which comprises 26 countries in the sub-Saharan region where the death rate resulted from the disease the highest[1].

The causative organisms of bacterial meningitis vary by age group. In neonates, a more significant number of bacterial meningitis cases result of group B *Streptococcus agalactiae*, *Escherichia coli* and *Listeria monocytogenes*. However, most cases of bacterial meningitis in children and adults are caused by *Streptococcus pneumoniae and Neisseria meningitidis* [3,4]. Although *Haemophilus influenzae* causes meningitis in all age groups, a greater number is usually seen in children under five (5) years of age [5,6].

Neisseria meningitidis is one of the most common causes of meningitis over the word. Large epidemics of the disease due to the meningococci have spread during the last decade

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throughout a large area of the meningitis belt of Africa and outside[7]. An estimated 500,000 cases and 50,000 deaths annually worldwide, is associated with *Neisseria meningitidis,* with children and young adults being the most vulnerable, according to the World Health Organization (WHO)[8]. *Neisseria meningitidis* can be classified based on the antigenicity of its surface polysaccharide capsule into thirteen (13) serogroups[9]. More than 90% of the globally invasive meningococcal infections are caused by serogroups, A, B and C. Serogroup A meningococci is implicated in a more significant number of the meningitis epidemics that occur in the African meningitis belt and China, but rarely in the industrialised countries [10,11,12,8,13]. Serogroup C is also implicated in the disease outbreak and occasional epidemics[14,15]. Serogroups B and C were responsible for endemic meningococcal meningitis with occasional occurrences as a result of serogroups, W135, Y and X[16,17]. However, serogroup W135 also causes endemic diseases in some African meningitis belt countries. The serogroup A epidemics in Africa's meningitis belt occur in a cycle of every 8-12years with each wave following a multi-year gradual increase in volume-decrease in volume of cases pattern[18]. The incidence of meningococcal meningitis, just like the other bacterial meningitis is seasonal dependent with peaks in the dry season (December-May) and decreases rapidly, even in times of major epidemic with the start of the rainy season[18,19].

Although meningococcal meningitis is endemic in various regions across the world, its burden is remarkable in 26 countries which make up the "meningitis belt" of sub-Saharan Africa, stretching from Senegal in the west to Ethiopia in the eastern part[20]. Meningococcal meningitis is hyperendemic in the region, with the number of incidence cases approaching 1000 per 100,000 population in the dry season. The annual epidemics in the region is broadly distributed across age groups. However, outside of epidemic situations, the highest incidence mostly occurs in young children[8].

The majority of meningococcal meningitis within the meningitis belt of the sub-Saharan Africa region between 1993 and 2012 was mainly due to serogroup A, the aetiological agent responsible for 80% of epidemics with approximately 1million cases and 100,000

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deaths[20]. Consequently, a monovalent meningococcal A conjugate vaccine (MenAfriVac) was developed and prequalified for children and adults aged 1-29years[21,22]. It was then used in a prophylactic vaccination campaign in Burkina Faso, Mali and Niger in 2010[23]. As a result of the vaccine introduction, by 2014, there were no cases due to serogroup A in those three countries[24] and other African countries such Ghana who implemented the vaccine programme. However, in the wake of decline of the disease due to serogroup A, serogroup W emerged as the predominant aetiological agent being responsible for 55% of confirmed cases in 2012 in the region[25]. Cases due to serogroup C are also in the upward trajectory in the sub-Saharan African region. 82.7% of 433 confirmed cases during the outbreak in Nigeria from December 2016 to June 2017 were due to serogroup C. It is considered the largest outbreak of meningococcal meningitis due to serogroup C in the word[26].

Although meningococcal meningitis incidence has been generally low in Europe, there have been some incidence increases over the past decade. The incidence in 2015 was 0.6 cases per 100,000 population in the European Union, a decline from the 0.7 in 2010. The highest number of cases per 100,000 population in 2015 were recorded in Lithuania (1.9), Ireland (1.5), the UK (1.4) and Malta (1.2)[27]. Even though the overall incidence is low, the emergence of certain serogroups is cause for worry. For instance, there has been increases in meningococcal disease incidence due to clonal complex variant of serogroup W in the UK. It accounted for 15% of cases during the 2013-2014 epidemiologic year compared to 1.8% during the 2008-2009 epidemiologic year[28], which affected mainly adults 45years and older. Cases, however, were distributed across all age group by 2011-2012. Also, there was a concern with the emergence of serogroup C in Italy in 2015 and 2016, with a substantial increase in cases (43cases) compared with those reported in 2013 (12) and 2014 (16). Of the 43 cases, 10 were fatal, and those affected were in the age range of 9 to 82 years[29]

In South America, the annual incidence of meningococcal meningitis varies considerably, ranging from less than 0.1 cases per 100,000 population in Bolivia, Cuba, Paraguay and Peru to almost 2 cases per 100,000 population in Brazil[30]. Serogroups B, C, Y and W are

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known to be responsible for cases in this region with serogroups B and C being responsible for most of the cases in the region. The emergence of serogroup W was also the cause of outbreaks in several countries within this region[30, 31].

Incidence rates in the Eastern Mediterranean are highest in Sudan and Yemen, with 13.26 and 4.74 cases per 100,000 population in 2006 and 2005, respectively. Serogroups A or W was responsible for most outbreaks in countries within the region, except for Israel, where outbreaks were due to serogroups B or C[32]. Recent surveillance data from Kuwait indicates an incidence rate of 0.5 per 100,000 population, with serogroups W and B being responsible for 32% of cases. Serogroup B was responsible for 34% of all cases in children aged 14 years and below, and serogroup W accounted for 40% of all adult cases. The 1987, 1989 and 2002 outbreaks in Kuwait were caused by serogroups, A,W and B respectively[33].

The northern part of Ghana lies within Africa's "meningitis belt" and has been experiencing bacterial meningitis outbreaks during the dry seasons, which is usually from December to May each year. Although only the northern part of Ghana lies within the African meningitis belt, other parts of the country are also at risk of attack, as evidenced in recent outbreaks where there were reported cases of serogroup W and C in the Brong Ahafo and the Ashanti regions, respectively in 2016. There have also been reported cases in the Central region and the Ashanti region during the recent outbreak (December – April 2020).

In 2010, Ghana recorded a total of 1164 cases of meningitis with 128 deaths, while in 2011 the country again had 790 cases with 104 deaths. The figure then increased to 956 cases in 2012 with 90 deaths and decreased to 454 cases and 41 deaths in 2013. There were 477 cases with 39 deaths and 315 cases with 33 deaths recorded in 2014 and 2015 respectively in the country[34]. A total of 280 and 241 cases were recorded in the 2016 and 2017, respectively. The predominant aetiological agent for the total cases of the 2016 and the 2017 was *Neisseria meningitidis*, with serogroup W being the most prevalent[35].

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Before 2012, serogroup A meningococcus was responsible for an estimated 80-85% of all cases of bacterial meningitis in the meningitis belt of the country (Ghana), with epidemics in every 7-14 years. However, after the mass prevention campaign of meningococcal serogroup A vaccine in the three northern regions in 2012, there has been a drastic decline in the number of cases attributable to it[34]. There have been zero cases attributable to it in recent outbreaks. Just like other countries within the African meningitis belt, the occurrence of meningitis outbreaks is now due to other meningococcal serogroups such as the serogroups W and X and Streptococcus pneumoniae which have been responsible for most of recent outbreaks[34-37].

To minimize the impact of early meningitis outbreaks, Ghana introduced *Haemophilus influenzae* b vaccine in 2002 and the 13-valent pneumococcal conjugate vaccine in 2012[38,39]. A serogroup A meningococcal conjugate vaccine (MenAfriVac) was introduced in 2012 through a mass vaccination campaign to reduce the burden of *N. meningitidis* outbreaks due to serogroup A, which was the most prevalent serogroup at the time. It was subsequently incorporated into the routine immunization program in 2016[38]. Despite the above interventions however, northern Ghana continued to experience meningitis outbreaks resulting from *Streptococcus pneumoniae* and serogroup W *N. meningitidis*. Given this, a reactive vaccination exercise of meningococcal polysaccharide ACW was carried out in the most affected districts in the Upper West region in 2016[38].

1.2 Problem statement

Despite the numerous interventions put in place by the Ministry of health with the support of international partners to reduce the burden of bacterial meningitis in the northern part of Ghana, where meningitis is endemic, yearly outbreaks still occur. These outbreaks are mainly caused by *Streptococcus pneumoniae* and *Neisseria meningitidis*. The *Neisseria meningitidis* cerebrospinal meningitis outbreaks were mainly caused by serogroups A before 2012, when its conjugate vaccine was introduced and subsequently incorporated into

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the routine immunization program in 2016, and serogroup W, which occasioned the mass vaccination campaign of the polysaccharide ACW vaccine in 2016 following its outbreak in the Upper West region the same year.

With the introduction of rt-PCR at the Tamale Public Health Laboratory in 2012, the aetiological agents of bacterial meningitis are being monitored. However, the actual trends in the non-groupable and the non-vaccine type serogroups of *Neisseria meningitidis* and the impact of the vaccination programs on the vaccine-preventable serogroups have not been adequately highlighted. Therefore, it is imperative that a study of this nature was carried out to help establish the trends in the various serogroups of *Neisseria meningitidis* in the meningitis belt of the country to determine whether there was an emerging threat from any of either the non-groupable or non-vaccine type *Neisseria meningitidis* serogroups, which had not posed any serious threat in the past, and to establish the influence of the vaccination programs introduced on vaccine preventable serogroups' trends.

1.3 Justification

Knowledge of the trends in the various serogroups of *Neisseria meningitidis* is very crucial in informing targeted public health interventions to bring the situation under control. For instance, if the trends in the vaccine types serogroups were still found to be taking an upwards trajectory, it would inform the need to review the vaccination regimen or improve upon the efficacy of the vaccines already available or procure a new vaccine altogether. It would also inform the need to undertake molecular study of the circulating serogroup strains to determine if the upward trend is due to mutation of the original strain based on which vaccines were developed. Again, if the upward trend was found to be in the non-vaccine type, it would inform the need for the appropriate authorities to start taking the necessary steps to educate the population at risk and procure vaccines and anti-microbials targeted at that particular serogroup to bring it under control before it becomes a public

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health threat. Moreover, Ghana can group only six (6) of the thirteen (13) known serogroups of *Neisseria meningitidis*. However, from time to time, some unknown or ungroupable serogroups are isolated. If an upward trend is seen in the ungroupable type, it would inform the need for further studies to identify those serogroups so that logistics are procured for effective surveillance.

1.4 General Objective

The primary objective of the study was to establish the trends in *Neisseria meningitidis* serogroups over five years in the meningitis belt of Ghana from 2016 - 2020

1.5 Specific Objectives

1. To determine the trends in *Neisseria meningitidis* serogroups in Ghana for the past five years.
2. To determine the trends of the various serogroups of *Neisseria meningitidis* in the reporting regions within the meningitis belt of Ghana
3. To determine the trends in the various gender and age groups in relation to the various *N. meningitidis* serogroups.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Bacterial Meningitis

Bacterial meningitis is a central nervous system disease caused by some bacterial types. It is characterized by inflammation of the membranes (meninges) covering the brain and spinal cord. The inflammation of the meninges put pressure on the brain and the spinal cord, causing potential life-threatening complications. It is usually preceded by respiratory illness or sore throat in adults and children[40,41, 42].

2.1.1 Signs And Symptoms of Bacterial Meningitis

Symptoms in adults and older children usually progress from irritability through confusion, drowsiness, and stupor, which may lead to coma. Dehydration is also common. Chills, weakness, stiff neck, loss of appetite and photophobia are other symptoms. Later symptoms may include paralysis of one side of the body (hemiparesis), loss of hearing, hydrocephalus (accumulation of fluid in the brain cavity), and other neurological disorders[40,41,43,44,42].

In infants in the age range of three (3) months and two (2) years, symptoms usually includes; fever, vomiting, irritability, convulsion and refusal of feeding. A high-pitched cry and bulging fontanel sometimes occur as a result. Cerebral fluid may accumulate inside the tough outer brain covering membrane several days after infection. The typical signs of meningitis in infants usually include; seizures, persistent fever and an increasing head size. Brain abscess or subdural pus accumulation may also occur. Accumulation of water in the brain (hydrocephalus), deafness and slowed mental and physical development are possible effects of bacterial meningitis on the central nervous system[40,41,43,44].

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2.1.2 Causes of Bacterial Meningitis

Bacterial meningitis is the most common cause of meningitis. Three (3) bacterial agents are responsible for about 80% of bacterial meningitis. These bacterial agents includes; *Hemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis* (Meningococcus).

Gram-negative bacteria such as E. coli, Klebsiella or Pseudomonas often cause bacterial meningitis in new-born infants. Streptococcus, *Staphylococcus aureus,* and *Listeria monocytogenes* are other causes of bacterial meningitis. Meningitis due to *Hemophilus influenza* (type B) usually occur in infants over a month old and young children. It normally does not occur in adults, however, it can occur in adults with underlining condition such head trauma or impaired immunity. Pneumococcal meningitis mostly occur in adults, especially those with sinusitus (inflammation of the mucous membranes lining the sinuses that open into the nose), mastoiditis (infection of the bone located in the ear), closed head injury, and pneumococcal pneumoniae[40,41,43,42].

Bacterial meningitis due to E. coli and Klebsiella-Enterobacter frequently occur after central nervous system trauma or surgery. Immune-compromised adults and new-borns may also be at risk. Staphylococcal meningitis may also occur after open head trauma, neurosurgery and blood poisoning as a result of endocarditis. Listeria meningitis, caused by *Listeria monocytogenes* infection, occurs mostly in new-borns, patients with chronic renal failure or adults on immunosuppressive drugs.

Bacterial agents that cause bacterial meningitis are thought to be carried in the throats of approximately 10% of the population. Of all the meningitis causing bacterial, *Hemophilus influenza* type B is the most common and accounts for almost half of all bacterial meningitis cases. Meningococcal and Pneumococcal meningitis are said to account for about twenty-seven (27) percent and eleven (11) percent of bacterial meningitis, respectively[42, 45].

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2.2 Meningococcal Meningitis

Meningococcal meningitis is bacterial meningitis resulting from the infection with *Neisseria meningitidis*. *Neisseria meningitidis* is solely a human Gram-negative diplococci which has a great genetic variety[46]. The genetic plasticity and the phenotypic diversity of the meningococcus are the hallmark of its evolution, as it acquired various genes from other species of Neisseria and other bacteria, such as *Hemophilus spp.* The meningococcus is a frequent coloniser of the nasopharynx and the oropharynx, however, it can also be found in other places such as the oral mucosa, the rectum and the urogenital tract[47]. The virulence of the meningococcus is related to its major outer membrane components, including the capsular polysaccharide, outer membrane proteins (pili, porins, Opa, Opc, meningococcal iron-acquiring proteins) and lipo-oligosaccharide (endotoxin)[47-49]. Thirteen (13) serogroups of Neisseria meningitidis have been characterized based on the different capsular polysaccharide structure, however, only six serogroups (A, B, C, W-135, X and Y) are known to cause life-threatening condition[49-51].

2.2.1 Global Epidemiology of Meningococcal Meningitis

Globally, the incidence of meningococcal disease changes greatly with respect to geographical location. An estimated 500,000-1,200,000 cases of invasive meningococcal disease are reported worldwide each year with 50,000-135,000 deaths[52, 53].

In Europe, North America and Australia, the disease incidence is estimated to range between 0.3 and 3 cases per 100,000 population[54]. However, during epidemics, incidence is estimated to range between 10-1,000 cases per 100,000 population in the “meningitis belt” of sub-Saharan Africa.

In many regions of the world, the epidemiology of meningococcal disease has substantially changed over time in recent years. Serogroup A had been the principal aetiological agent

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for invasive meningococcal infection in Europe before and during the World Was I and II. Serogroup B has been the prevalent serogroup since 1970 in Europe and 1980 in South America. However, epidemic outbreaks due to serogroups W-135 and Y have emerged more recently during the XXIst century. Besides the change in the age classes affected by invasive meningococcal disease, there has been an increase in the incidence of serogroup Y in the elderly and a decreases of serogroup C incidence in adolescents. The trends in the epidemiology of invasive meningococcal meningitis has largely remain unchanged in Africa, with serogroup A being the most prevalent serogroup. However, serogroups X and W-135 have had a significant impact in terms of morbidity and mortality in recent times[55].

The incidence of meningitis caused by serogroup A *Neisseria meningitidis* in Africa is estimated to be 10-20 cases per 100,000 population annually. During epidemic outbreaks in the dry seasons, an attack rate is estimated to be more than 1,000 cases per 100, 000 population. In Latin America however, the incidence ranges between 0.1 cases per 100,000 population in Mexico to 2 cases per 100,000 population in Brazil, with the predominance of serogroups B and C[56]. The incidence in Australia is estimated to be more than 3 cases per 100,000 population. The epidemiological burden of meningococcal infection is not well defined in Asia. However, serogroup A is considered the most prevalent. Five (5) serogroups (A, B, C, Y and W-135) have been reported in Asia with regional variation[57].

In most countries in America and Europe, there is low level endemicity of meningococcal disease. Twenty-nine (29) European countries (27 EU countries and Norway and Iceland), in 2011 reported 3,808 confirmed cases of the disease[58]. The global notification rate has been estimated to be 0.77 cases per 100,000 population, with serogroup B being the most relevant, accounting for 73.6% of cases, followed by serogroup C, 14.4% and serogroup Y making up 8.2%. The disease incidence sustained by serogroup B in Europe accounts for 0.5 cases per 100,000 population. Italy reports the lowest incidence rate with 0.25 cases per 100,000 population[59].

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The regional or geographical distribution of the main serogroups of the *Neisseria meningitidis* bacteria are shown in figure 1 below.

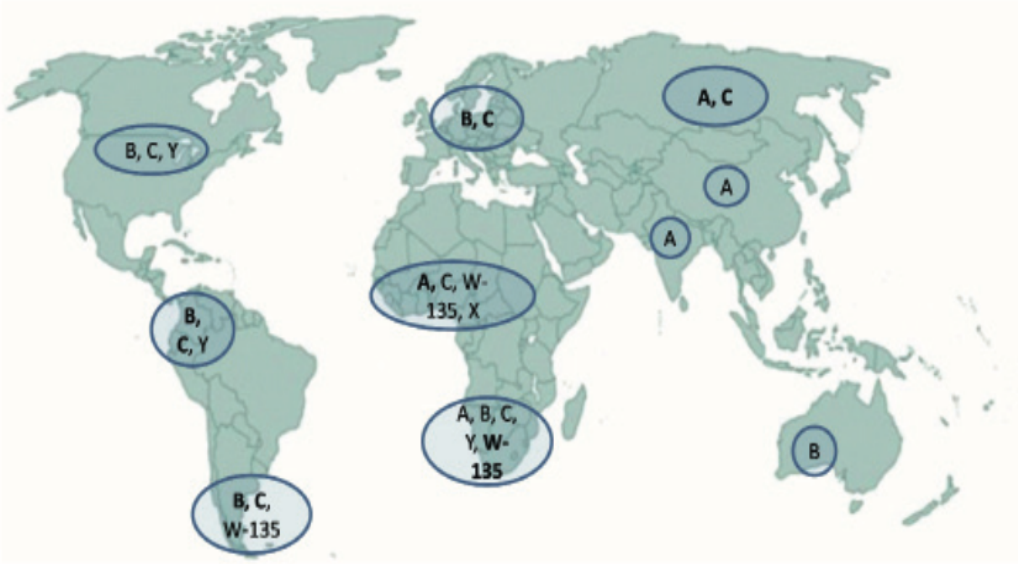

Figure 1: Global distribution of *Neisseria meningitidis* serogroups (Source: Journal of preventive medicine and hygiene 2015)

2.2.1.1 Epidemiology of Meningococcal Meningitis in Africa

Meningococcal meningitis incidence rate in sub-Saharan Africa greatly exceeds those of the other regions in the world[60]. The disease epidemics in the meningitis belt of Africa usually occurs in the dry season from January through to June when the incidence rate can go as high as 1,000 cases per 100,000 population. The rate of endemic disease remains relatively high at 10-25 cases per 100,000 population in the periods between outbreaks. Between 1990 and 2010, the predominant serogroup associated with outbreaks in the region was serogroup A[61]. The high incidence led to the development of a serogroup A

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polysaccharide conjugate vaccine (MACV) that was administered in 2010 to nearly 100% of the population between the ages of 1-29 years in Burkina Faso. The vaccination campaign with MACV has since been expanded to a number of countries within the meningitis belt of the region with high coverage rate[20, 62, 63]. Before the introduction MACV in 2010, the case fatality ratio (CFR) among countries in the African meningitis belt ranged from 4% in Mali to 26% in Benin[61]. However, since the introduction of the vaccine, the overall CFR in surveillance countries has been relatively stable at 8.5%, 9.1% and 9.1% in 2012, 2013 and 2014, respectively[20, 62, 63] .

With the wide spread use of MACV, there has been a reported overall reduction in epidemic activity due to serogroup A among countries in the meningitis belt of the region. During 2012 meningitis season, Burkina Faso reported more cases of meningitis than in the 2011 season. This was largely due to the increase in serogroup W cases[64]. However, in the region of Chad where the vaccine was not used, serogroup A was still predominant[65], demonstrating a shift in serogroups in communities in countries with a successful vaccination program. Among meningitis cases in 2012 through to 2014 from which the serogroup had been determined, serogroup W had been the most common serogroup now with rates, 76%, 72% and 81% in 2012, 2013 and 2014 respectively[20, 62, 63].

There has been a documentation of serogroup X meningococcal meningitis in a number of African countries. Outbreaks due serogroup X was reported in Niger, Kenya and Uganda in 2006[66]. Although the incidence rate of meningococcal meningitis due serogroup X in the region is relatively low, the number of cases, as well as the lack of an effective vaccine, calls for the need for continued surveillance of the disease associated with this serogroup in the African meningitis belt.

The figure below illustrates the “Meningitis belt” of the sub-Saharan Africa region showing areas or countries with frequent epidemics of meningococcal meningitis.

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Figure 2 : Countries with frequent epidemics of meningococcal meningitis in sub-Sahara Africa (source: World Health Organization. International Travel and Health. Geneva, Switzerland: 2012)

2.2.2 Incidence Among Age Groups.

Meningococcal disease incidence is highest in infants under one (1) year of age and remains relatively high until about 5 years of age. Although the incidence tends to decrease in older children, it usually spikes later during adolescence and young childhood. Incidence again peaks off in older adults. This pattern has been reported both at the regional and country levels[58, 60, 67-70]. The CFR of invasive meningococcal disease is sometimes higher in infants than in older children. However, it is routinely highest in adults aged 65 years and older[71, 72].

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Trends are also observed when examining the prevalence of the various serogroups of *Neisseria meningitidis.* In North America and Europe, serogroup B is the most common serogroup associated with invasive meningococcal disease in infants, with incidence also routinely spiking during adolescence and young childhood[58, 67, 68, 73-75]. It has been observed recently through surveillance data that serogroup B is the causative agent for at least 70% of IMD cases in all age groups up to 24 years of age in Europe[58].

Again, in Europe, a substantial increases in serogroup Y IMD in under 1 year, 1-4 years and 25-49 years age group was observed between 2008 and 2011. However, in England the highest incidence of serogroup Y was observed in those aged 15-19 years, 45-64 years and those 65 years and older. In the United States, serogroup Y IMD is typically seen in older individuals, although cases in infants have become more common in recent years[58, 76].

Finally, the most relevant rate of invasive meningococcal disease, particularly in children younger than 5 years of age, is related to serogroup B, followed by serogroup C. the notification rate of serogroup B infection in children under 1 year of age in 2012 was reported to be three-fold higher than those registered in the age group 1-4 years. The highest rate of meningococcal infection due to serogroup C has been reported in young adults and adults 25-44 years of age[77].

2.2.3 *Neisseria meningitidis* Carriage and the Effects of Vaccine

Neisseria meningitidis is one of the common components of the human nasopharyngeal microbiota and its carriage is an important precursor to the development of invasive meningococcal disease. While invasive meningococcal disease incidences are highest in infants and young children, carriage rates usually peaks in late adolescent or young adulthood[78, 79]. Current studies have shown carriage rate of 4.5% in infants, 23.7% in people age 19 years and 7.8% in those aged 50 years in countries such as those in Europe where the predominant serogroups are B and C[79]. In the meningitis belt of Africa, studies have

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shown carriage rates of *Neisseria meningitidis* to be 1.8% in infants, 4.9% for those aged between 5-14 years and 2.6% for those aged 30 years or older[78]. In 2012, a study among university students in Chile showed a carriage rate of 4%[80]. In the U.S. states of Georgia and Maryland, studies in 2006-2007 showed a relatively low carriage rates of 3.2%-4.0% of the bacteria[81]. This highlights the fact that variation in carriage may be observed in different geographical locations.

The prevention of meningococcal serogroup C carriage and its subsequent herd immunity leading to a reduction of invasive meningococcal disease in the unimmunized population is an important benefit of immunization programs. Reduction in *Neisseria meningitidis* carriage after immunization appears limited to the polysaccharide-conjugate formulations and is hardly seen with polysaccharide only formulation[82]. The administration of a meningococcal A/C polysaccharide vaccine during an outbreak in Brazil had no effect on the overall carriage rate. However, meningococcal conjugate C vaccine have routinely demonstrated serogroup specific reduction in carriage. The administration of serogroup C conjugate vaccine in the United Kingdom, for instance, led to a significant reduction in serotype C carriage in university students for up to 2 years after the vaccination[83]. On the other hand, the carriage rate of meningococcal serogroup Y has been increasing in a cohort study of university students in the United Kingdom[84]. A carriage studies in Burkina Faso after 2 years of widespread use of serogroup A conjugate vaccine have shown that carriage of serogroup A has been nearly eliminated[84].

2.2.4 Recent Outbreaks of *N. meningitidis* in the African Meningitis Belt

A total 17 outbreaks of meningococcal meningitis have been reported in countries within the meningitis belt of Africa who have implemented the MACV program between 2011 and 2017. These outbreaks were reported in eight (8) countries with the total number of cases being 31,786. From 2011 to 2014, only one outbreak per year were reported.

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However, from 2015 to 2017, 3 to 6 outbreaks were reported per year, with almost all occurring during the epidemic seasons with the exception of one outbreak in a refugee camp in Ethiopia[85].

The country which suffered the most was Nigeria, which reported five (5) outbreaks with a total of 17,375 suspected cases in 55 epidemic districts. This was followed by Niger which had four (4) outbreaks with 9,343 cases in 21 epidemic districts. Burkina Faso experienced a large outbreak in 2012 with 2,372 cases in 13 epidemic districts but no further outbreaks were subsequently reported. One outbreak was reported in Togo in 2016[86] and 2 outbreaks in 2017. Benin, Cameroun, Ethiopia and Ghana have each reported one outbreak within the period. The size of the outbreaks ranged from 18 cases which were reported in the refugee camp in Ethiopia to 14,542 cases reported in Nigeria in 2017. There were two outbreaks which were deemed special, where two confirmed cases in a week represented an epidemic. One was reported in a refugee camp in Ethiopia in 2015 and the other was in a prison in Cameroun in 2017. The number of reported cases in these two outbreaks; 18 and 25, respectively were considered the lowest within the period, with case fatality ratio (CFR) of 0% and 32% respectively. The average CFR for all the outbreaks within the period, with the exception of the two special situations, was 6% (2% - 11%). The total cases reported with those 17 outbreaks period constituted 45% of all cases reported after the introduction of MACV, ranging from 3% in 2011 to 83% in 2017[85].

Serogroup C was the predominant meningococcal serogroup responsible for 11 of the 17 outbreaks (Benin, Cameroun, Ethiopia, Niger and Nigeria) within the period, involving 78 epidemic districts with 26,710 cases reported. Serogroup W was predominant in the other six (6) outbreaks (Niger in 2011, Burkina Faso, Ghana and Togo), involving 35 epidemic districts with 4,935 cases[85]. Remarkably, there were no outbreaks resulting from serogroup A. Although, serogroup X was not the predominant serogroup in any of the outbreaks, its proportion was sizeable in the outbreak in Togo in 2017, where it constituted 37% of the cases identified.

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CHAPTER THREE

3.0 METHODOLOGY

3.1 Study Population

The study population included suspected cerebrospinal meningitis patients in the Northern zone of Ghana diagnosed within the study period. PCR confirmed results of these patients at the Tamale Public Health Laboratory within the study period were included in the study.

3.1.1 Inclusion Criteria

- Only data of suspected patients who were confirmed residents of Ghana at the time of the suspected disease condition were considered for the study.
- All PCR confirmed cases of suspected cerebrospinal meningitis cases within the study period were included in the study.
- All PCR confirmed data of *Neisseria meningitidis* positive cases with results for serogroups were included in the study.

3.1.2 Exclusion Criterial

- Cases with incomplete data, including demographic data such as age and gender or sex, and or region of residence missing were excluded from the study
- All cases which were not confirmed by PCR were excluded from the study
- All *Neisseria meningitidis* positive cases without results for serogroups were also excluded from the study.

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3.2 Study Site

The study's data were collected at the Tamale Public Health Laboratory, which is situated in the northern region of Ghana. The Tamale Public Health Laboratory is designated as the national reference laboratory for confirmatory diagnosis of cerebrospinal meningitis caused by bacterial pathogens in Ghana. Tamale is located in the center of the Northern Region with an approximated land size of 646.90180sqkm with a population of 371,351inhabitants. The facility is equipped with a Real-time PCR for the species and serogroups and serotypes identification of pathogens of bacterial meningitis. Cerebrospinal fluid (CSF) sample of all suspected meningitis patients from all parts of the country, especially the regions in the northern zone of the country which fall within Africa's meningitis belt, are sent to the facility for laboratory confirmation via PCR

3.3 Study Period

The study was a retrospective study that relied on data collected over five years, spanning from 2016 to the first half of 2020. Complete data of all suspected cerebrospinal meningitis cases confirmed by PCR at the Tamale Public Health Laboratory over this period were collected.

3.4 Sample size

A predefined or calculated sample size was not set for this study. It included all reported cases of cerebrospinal meningitis confirmed by PCR at the Tamale Public Health Laboratory within the set study period.

3.5 Data Source, Collection and Management

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The data for this study was sourced from the Tamale Public Health Laboratory. The patients' demographic data were obtained from the case investigation forms accompanying the cerebrospinal spinal (CSF) samples for laboratory confirmation.

The demographic data of patients collected included age, sex and region of residence. The PCR confirmed laboratory data collected included results for both speciation and serogroups of all *Neisseria meningitidis* positive and speciation only for all other cases within the period.

For the purposes confidentiality and security of the patients, names and epidemiological numbers were unlinked anonymously. The data collected was entered into a password-protected database with access to it only by myself and my thesis supervisor.

3.6 Sample Analysis for Aetiological Agents Identification

3.6.1 CSF Culture

Three samples of CSF of each suspected case of cerebrospinal meningitis were received at the Tamale Zonal Public Health Laboratory for analysis. Two, each of at least 1ml of the CSF in dry cryotubes and another 1ml in trans isolate (TI) medium. TI medium samples were used for culture and sensitivity testing. Samples in the cryotubes were used for the PCR analysis and Latex Agglutination tests, Gram's staining and physical and microscopic examinations. All CSF sample received were processed using standard microbiological and molecular techniques.

All CSF samples for culture were inoculated on Chocolate agar, Columbia sheep blood agar and MacConkey agar using sterile disposable loops. The inoculums were streaked out on the plates and incubated at 37°C (5% CO2) for 18-24 hours. The plates were observed after the incubation period for bacterial growth or colony formation. Morphological characteristics, lactose fermentation and haemolysis on the various media and Gram's

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reaction were used to categorize the bacteria into Gram positives and negatives. All Gram-positive bacteria were identified following a biochemical chart. Optochin disc was used to differentiate *Streptococcus pneumoniae* from other alpha haemolytic streptococcus species which are resistant to the Optochin disc.

3.6.2 Antimicrobial Susceptibility Testing.

Antimicrobial susceptibility testing on all culture positive samples was performed using the Kirby-Bauer disc diffusion method. Pure growth of bacteria less than 24 hours old was used to set up the susceptibility testing. The following antibiotics were used for the susceptibility testing; amoxiclav (amoxicillin & clavulanic acid; 20/10µg), ceftriaxone (30 µg), azithromycin (15 µg), amikacin (30 µg), meropenem (10 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), gentamycin (10 µg), ceftazidime (30 µg) and cefotaxime (30 µg). The antibiotics were selected based on the CLSI guidelines.

The testing was done by inoculating pure culture directly in 0.85% saline and adjusted to a turbidity of 0.5 McFarland standard. The inoculum was streaked uniformly on the entire plate of Mueller-Hinton agar (BD, USA) using sterile cotton buds and incubated overnight at 37°C. The plates were read after overnight incubation and the zone diameters interpreted using the zone diameter recommendation from CLSI.

3.6.3 Confirmation with Real-Time PCR (rt-PCR)

All the sample were confirmed using Direct Real-Time Polymerase Chain Reaction (PCR) technology. A triplex detection method was used for species and *Streptococcus pneumoniae* serotypes identification using Cy5, HEX and FAM as the differentiating dyes.

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The serogroups of *Neisseria meningitidis* and *Haemophilus influenzae* were identified via monoplex detection, using FAM and ROX dyes, with the ROX as the reference dye.

With the triplex detection, a single Master Mix was prepared and used for the simultaneous detection of *Neisseria meningitidis, Streptococcus pneumoniae* and *Haemophilus influenzae* species. The constituents of the Master Mix included; primers (both forward and reverse) and probes of all the species being tested in equal volumes, PCR grade water and Multiplex Quanta. The ratios were 12.5 µL:7.5 µL:1 µL for Master Mix, PCR grade water and primers and probes respectively, for a sample.

All samples which tested positive for *Neisseria meningitidis* were then selected, and their serogroups identified using the monoplex detection method. A Master Mix was prepared for each of the six serogroups being tested for. Each master mix's constituents included the primers (both forward and reverse) and probes of the serogroup of interest, PCR grade water and a monoplex Quanta with Low ROX in the same ratios as for the triplex detection.

Reaction templates were prepared for each reaction depending on the number of samples being tested, and the master mix was prepared accordingly. The master mix and samples were added to the PCR reaction plate wells in a ratio of 23µL:2µL, respectively. The controls were run simultaneously with sample. However, when new dilutions of primers and probes were made, they were controlled before testing of patient samples.

The prepared reaction plate with sample reagents mix was loaded into the Agilent AriaMx Real-Time PCR analyser for amplification and detection at a 50-cycle for a time of 01:42:47. The cycling conditions included a first step of 95 degree Celsius for 15seconds and a second step of 60 degree Celsius for 1minute for the amplification segment. The amplification curves and Cq values for the sample at the end of the reaction cycles were analysed, and the results were interpreted. Samples with Cqs ≤ 34 were interpreted as positive, those between 34 and 35 were considered equivocal and were repeated and those with no Cqs or Cqs greater than 35 were considered negative.

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3.7 Statistical Analysis

The data collected were entered into the Statistical Package for Social Sciences (SPSS) software program and analysed. Differences between discrete variables were analyzed using Jonckheere-Terpstra Non-Parametric Test. A p-value <0.05 was considered statistically significant

3.8 Ethical Consideration

The study's ethical approval was obtained from the Yonsei University's Institutional Review Board (IRB) of Severance Hospital with the approval number IRB No: Y-2020-0105. Permission was also obtained from the Ghana Health Service Northern Regional Directorate to use the data on meningitis from the Public Health Laboratory of the region for the purpose of academic publications.

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CHAPTER 4

4.0 RESULTS

4.1 General and Sociodemographic Characteristics of the study population

A total of 2426 suspected cases were included in the study. Of this number, 236, 747, 538, 634 and 271 were tested in 2016, 2017, 2018, 2019 and 2020 respectively.

Of the total number tested within the study period, 52.6% were males and 47.4% were female (as shown in Table 1). Regarding the regional distributions, 44.4% were from the Upper West region, 37.0% from the Northern region, 12.2% from the Upper east region, 6% from the Brong Ahafo Region (Bono East, Ahafo and Bono) and 0.3% from the Ashanti region (shown in Table 1). Concerning the age-group distribution, of all the suspected cases, 2.9% were under the age of one year, 4.9%, 9.8%, 12.7%, 13.4%, 18.3%, 18.4% and 19.5% were within the age groups; 65years and older, 45-64years, 1-4years, 11-15years, 5-10years, 24-44years and 16-23years, respectively (as shown Table 1).

4.2 All Suspected Cases by Causal or Aetiological Agents of Bacterial Meningitis

Of the total suspected cases, 63.4% were negative for all the aetiological agents tested for by PCR (as shown in table 1). 19.1%, 16.3% 1.2% and approximately 0.0% tested positive for *Streptococcus pneumoniae*, *Neisseria meningitidis, Haemophilus influenzae* and *streptococcus group B* respectively (shown in table 1).

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Table1: Sociodemographic distribution and aetiological agents of all suspected cases of bacterial cerebrospinal meningitis (CSM) cases confirmed by Polymerase Chain Reaction (PCR)

	2016 N=236	2017 N=747	2018 N=538	2019 N=634	2020 N=271	Total N=2426
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Aetiological Agents						
N. meningitidis	94(39.3)	120 (16.1)	59 (11.0)	56 (8.8)	66 (24.4)	395 (16.3)
S. pneumoniae	51(21.6)	159 (21.3)	100 (18.6)	96 (15.1)	57 (21.0)	463 (19.1)
H. influenzae	3 (1.3)	8 (1.1)	8 (1.5)	4 (0.6)	5 (1.8)	28 (1.2)
Streptococcus B	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	1(0.0)
Negative	88 (37.3)	460 (61.6)	370 (68.8)	478 (75.4)	143 (52.8)	1539 (63.4)
Age Groups (Years)						
Under 1	4 (1.7)	29 (3.9)	16 (3.0)	19 (3.0)	3 (1.1)	71 (2.9)
1-4	41 (17.4)	113 (15.1)	62 (11.5)	54 (8.5)	37 (13.7)	307 (12.7)
5-10	53 (22.5)	145 (19.4)	90 (16.7)	99 (15.6)	58 (21.4)	445 (18.3)
11-15	35 (14.8)	98 (13.1)	63 (11.7)	80 (12.6)	49 (18.1)	325 (13.4)
16-23	48 (20.3)	140 (18.7)	108 (20.1)	130 (20.5)	48 (17.7)	474 (19.5)
24-44	34 (14.4)	123 (16.5)	116 (21.6)	125 (19.7)	48 (17.7)	446 (18.4)
45-64	17 (7.2)	58 (7.8)	52 (9.7)	91 (14.4)	20 (7.4)	238 (9.8)
65 and Older	4 (1.7)	41 (5.5)	31 (5.8)	36 (5.7)	8 (3.0)	120 (4.9)

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Regions						
Ashanti	0 (0.0)	8 (1.1)	0 (0.0)	0 (0.0)	0 (0.0)	8 (0.3)
Brong Ahafo	0 (0.0)	54 (7.2)	0 (0.0)	69 (10.9)	9 (3.3)	132 (5.4)
Bono East	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (3.7)	10 (0.4)
Bono	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (1.8)	5 (0.2)
Northern	122 (51.7)	265 (35.5)	204 (37.9)	210 (33.1)	97 (35.8)	898 (37.0)
Upper East	57 (24.2)	90 (12.0)	57 (10.6)	74 (11.7)	17 (6.3)	295 (12.2)
Upper West	57 (24.2)	330 (44.2)	277 (51.5)	281 (44.3)	133 (49.1)	1078 (44.4)
Sex/Gender						
Male	127 (53.8)	412 (55.2)	274 (50.9)	315 (49.7)	147 (54.2)	1275 (52.6)
Female	109 (46.2)	335 (44.8)	264 (49.1)	319 (50.3)	124 (45.8)	1151 (47.4)

N-Total sample size, n-cases, %-percentage

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Table 2: Sociodemographic Distribution of the various serogroups of *Neisseria meningitidis* positive cases confirmed by PCR

	B N=2	C N=2	NG N=12	W N=282	X N=97	Total N=395
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Regions						
Brong Ahafo	0 (0.0)	0 (0.0)	0 (0.0)	5 (1.8)	0 (0.0)	5 (1.3)
Northern	1 (50.0)	0 (0.0)	6 (50.0)	183 (64.9)	15 (15.5)	205 (51.9)
Upper East	0 (0.0)	0 (0.0)	3 (25.0)	40 (14.2)	40 (41.2)	83 (21.0)
Upper West	1 (50.0)	2 (100.0)	3 (25.0)	54 (19.1)	42 (43.3)	102 (25.8)
Age Group						
Under 1	0 (0.0)	0 (0.0)	0 (0.0)	8 (2.8)	0 (0.0)	8 (2.0)
1-4	0 (0.0)	0 (0.0)	1 (8.3)	58 (20.6)	9 (9.3)	68 (17.2)
5-10	0 (0.0)	1 (50.0)	4 (33.3)	82 (29.1)	44 (45.4)	131 (33.2)
11-15	1 (50.0)	0 (0.0)	4 (33.3)	50 (17.7)	23 (23.7)	78 (19.7)
16-23	1 (50.0)	1 (50.0)	1 (8.3)	56 (19.9)	15 (15.5)	74 (18.7)
24-44	0 (0.0)	0 (0.0)	2 (16.7)	18 (6.4)	3 (3.1)	23 (5.8)
45-64	(0.0)	0 (0.0)	0 (0.0)	10 (3.5)	3 (3.1)	13 (3.3)
Sex/Gender						
Male	1 (50.0)	2 (100.0)	7 (58.3)	156 (55.3)	57 (58.8)	223 (56.5)
Female	1 (50.0)	0 (0.0)	5 (41.7)	126 (44.7)	40 (41.2)	172 (43.5)

B, C, W and X- *Neisseria meningitidis* Serogroups B, C, W and X, respectively. NG-Non-groupable serogroups

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4.3 Sociodemographic Effect and serogroup Distribution

Of the 395 cases tested positive for *Neisseria meningitidis*, 282, 97, 12, 2,2 were for serogroups W, X, NG, B and C, respectively (as shown in Table 2). Of the total, 56.5% were males and 43.5% were females. 51.9%, 25.8%, 21.0% and 1.3% of the *Neisseria meningitidis* cases were from the Northern region, Upper West region, Upper East region and the Brong Ahafo region respectively. With respect to the age group distributions, 33.2%, 19.7%, 18.7%, 17.2%, 5.8%, 3.3% and 2.0% were in the age groups, 5-10years, 11-15years, 16-23years, 1-4years, 24-44years, 45-64yeas and under 1 year respectively (as shown in table 2).

Table 3: Distribution/Trend of the various *Neisseria meningitidis* serogroups confirmed by PCR by year

	2016 N=94	2017 N=120	2018 N=59	2019 N=56	2020 N=66	Total N=395	
***N. meningitidis* Serogroups**	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	p-value
B	0 (0.0)	1 (0.8)	1 (1.7)	0 (0.0)	0 (0.0)	2 (0.5)	0.78
C	1 (1.1)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.5)	0.05
NG	0 (0.0)	0 (0.0)	5 (8.5)	5 (8.9)	2 (3.0)	12 (3.0)	0.21
W	92 (97.9)	116 (96.7)	36 (61.0)	28 (50.0)	10 (15.2)	282 (71.4)	0.01
X	1 (1.1)	2 (1.7)	17 (28.8)	23 (41.1)	54 (81.8)	97 (24.6)	0.01

B, C, W and X- *Neisseria meningitidis* Serogroups B, C, W and X, respectively. NG-Non-groupable serogroups.

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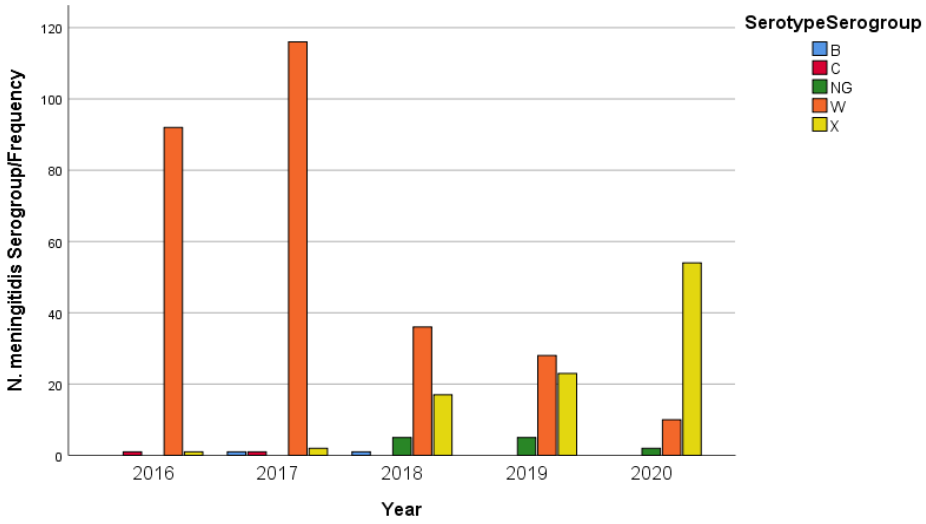

Figure 3: Trends in *Neisseria meningitidis* serogroup

4.4 Yearly Trend in *Neisseria Meningitidis* Serogroups from 2016-March 2020

There is a significant upward trend of serogroup X (p=0.01) and downward trend of the serogroup W (p=0.01) and serogroup C (0.05). The serogroup B is also showing a downward trend and the NG serogroups are in the upward trajectory. However, the yearly changes in the serogroups; B and NG are insignificant as shown in table 3 (with p-values; 0.78, and 0.21, respectively).

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Tables 4: Distribution/Trend of the various *Neisseria meningitidis* serogroups confirmed by PCR by Age Group and year

		2016	2017	2018	2019	2020	Total	
		N=94	N=120	N=59	N=56	N=66	N=395	
Age Group	serogroup	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	p-value
Under 1	W	2(100.0)	2(100.0)	3(100.0)	1(100.0)		8(100.0)	0.16
1-4	NG	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (11.1)	1 (1.5)	0.16
	W	21 (100.0)	25 (100.0)	6 (75.0)	5 (100.0)	1 (11.1)	58 (85.3)	0.16
	X	0 (0.0)	0 (0.0)	2 (25.0)	0 (0.0)	7 (77.8)	9 (13.2)	0.17
5-10	C	1 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.8)	0.16
	NG	0 (0.0)	0 (0.0)	2 (10.0)	1 (6.7)	1 (3.7)	4 (3.1)	0.45
	W	22 (91.7)	44 (97.8)	10 (50.0)	4 (26.7)	2 (7.4)	82 (62.6)	0.05
	X	1 (4.2)	1 (2.2)	8 (40.0)	10 (66.7)	24 (88.9)	44 (33.6)	0.05
11-15	B	0 (0.0)	1 (5.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)	0.48
	NG	0 (0.0)	0 (0.0)	1 (8.3)	3 (23.1)	0 (0.0)	4 (5.1)	0.41
	W	16 (100.0)	18 (94.7)	7 (58.3)	6 (46.2)	3 (16.7)	50 (64.1)	0.01
	X	0 (0.0)	0 (0.0)	4 (33.3)	4 (30.8)	15 (83.3)	23 (29.5)	0.07
16-23	B	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	1 (1.4)	1.00
	C	0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.4)	0.48
	NG	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	1 (1.4)	1.00

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	W	17 (100.0)	20 (90.9)	9 (69.2)	7 (50.0)	3 (37.5)	56 (75.7)	0.01
	X	0 (0.0)	1 (4.5)	2 (15.4)	7 (50.0)	5 (62.5)	15 (20.3)	0.01
24-44	NG	0 (0.0)	0 (0.0)	1 (50.0)	1 (25.0)	0 (0.0)	2 (8.7)	0.78
	W	9 (100.0)	5 (100.0)	1 (50.0)	2 (50.0)	1 (33.3)	18 (78.3)	0.04
	X	0 (0.0)	0 (0.0)	0 (0.0)	1 (25.0)	2 (66.7)	3 (13.0)	0.05
45-64	W	5 (100.0)	2 (100.0)	0 (0.0)	3 (75.0)	0 (0.0)	10 (76.9)	0.12
	X	0 (0.0)	0 (0.0)	1 (100.0)	1 (25.0)	1 (100.0)	3 (23.1)	0.12

B, C, W and X- *Neisseria meningitidis* Serogroups B, C, W and X, respectively. NG-Non-groupable serogroups

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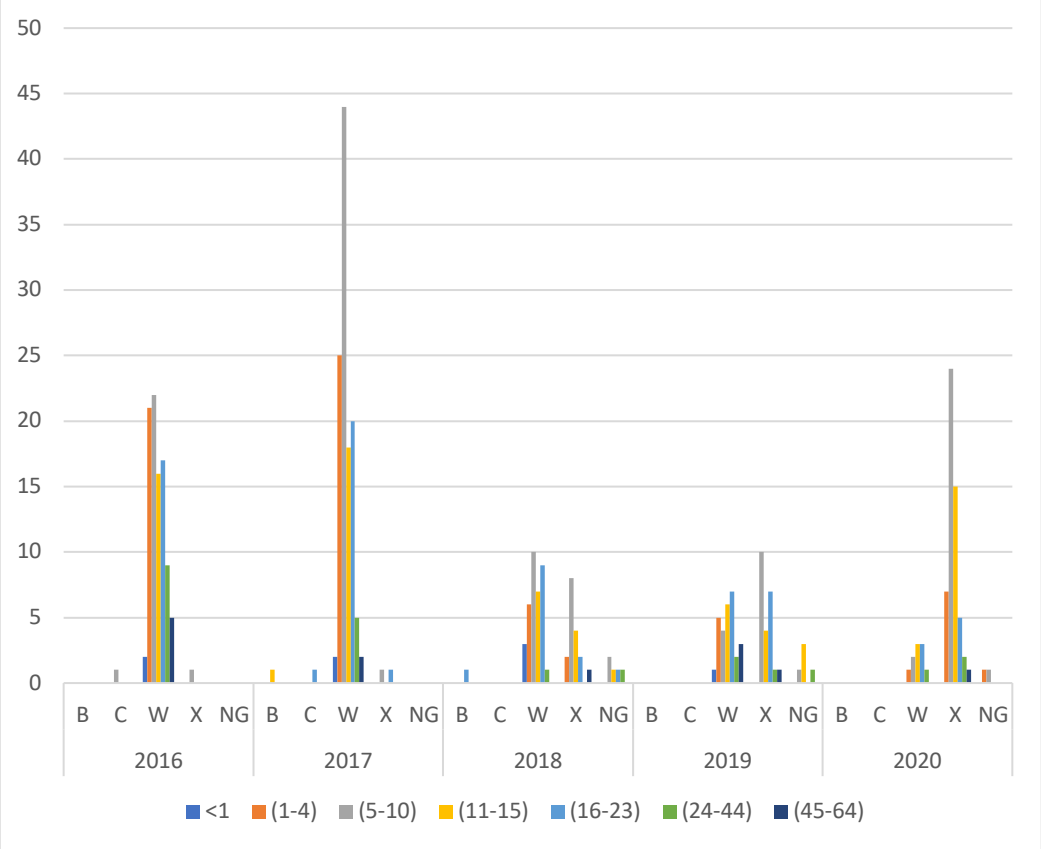

Figure 4: *N. meningitidis* Serogroups trends in Different Age Group (2016-2020)

4.5 Serogroup Trends In Different Age Group

In 2016 (as shown in Table 4), all the *Neisseria meningitidis* cases within the age groups under1 year and 1-4years were serogroup W. In the age group, 5-10years, 91.7%, 4.2% and 4.2% of the cases were serogroups, W, X and C respectively. For the age groups 11-15years, 16-23years, 24-44years and 45-64years, 100% of the cases were serogroup W respectively. In 2017, 100% of cases within the age groups, 1-4years, 24-44years, 45-64years and under 1year were serogroup W. In the same year, in the group 5-10years,

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97.8% of cases were serogroup W and 2.2%, serogroup X. 94.7% and 90.9% of cases in age group 11-15years and 16-23years, respectively were serogroup W and 5.3% and 4.5% for serogroups B and X respectively. Of the total cases in 2018, 8 cases were for people within the age group 1-4, of which 75% were serogroup W and 25% were serogroup X. 3 cases were for the age group under 1year of which all were serogroup W. 50%, 40% and 10% were serogroups W, X and NG respectively for 5-10years age group. As can be seen from Table 4, there have been downward and upward trends of serogroups B, C, W and NG, and the serogroup X respectively. However, the yearly changes of the serogroups B, C, and NG were insignificant across all age groups. The yearly changes of the serogroup W, however, are significant across almost age groups, and that of the serogroup X was significant in only the age groups; 5-10years, 11-15years, 16-23years and 24-44years.

Table 5: Distribution/Trend of the various *Neisseria meningitidis* serogroups confirmed by PCR by Region of residence year.

Region	Serogroup	2016 N=94 n (%)	2017 N=120 n (%)	2018 N=59 n (%)	2019 N=56 n (%)	2020 N=66 n (%)	Total N=395 n (%)	p-value
Brong Ahafo	W		4 (100.0)		1 (100.0)		5 (100.0)	1.00
Northern	B	0 (0.0)	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	1 (0.5)	1.00
	NG	0 (0.0)	0 (0.0)	4 (12.9)	2 (11.1)	0 (0.0)	6 (2.9)	0.78
	W	59 (100.0)	77 (97.5)	23 (74.2)	15 (83.3)	9 (50.0)	183 (89.3)	0.05
	X	0 (0.0)	2 (2.5)	3 (9.7)	1 (5.6)	9 (50.0)	15 (7.3)	0.05
Upper East	NG	0 (0.0)	0 (0.0)	0 (0.0)	3 (11.5)	0 (0.0)	3 (3.6)	0.48

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	W	14 (93.3)	24 (100.0)	1 (7.7)	1 (3.8)	0 (0.0)	40 (48.2)	0.05
	X	1 (6.7)	0 (0.0)	12 (92.3)	22 (84.6)	5 (100.0)	40 (48.2)	0.42
Upper West	B	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.00	1 (1.0)	0.48
	C	1 (5.0)	1 (7.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.0)	0.16
	NG	0 (0.0)	0 (0.0)	1 (6.7)	0 (0.0)	2 (4.7)	3 (2.9)	0.41
	W	19 (95.0)	11 (84.6)	12 (80.0)	11 (100.0)	1 (2.3)	54 (52.9)	0.33
	X	0 (0.0)	0 (0.0)	2 (13.3)	0 (0.0)	40 (93.0)	42 (41.2)	0.17

B, C, W and X- *Neisseria meningitidis* Serogroups B, C, W and X, respectively. NG-Non-groupable serogroups

4.6 Regional Trends of Serogroups

In 2016, 100%, 93% and 95% of positive cases recorded in the Northern, Upper East and Upper West regions respective were serogroup W (as shown in Table 5). In 2017, 100%, all the cases in Upper East and Brong Ahafo regions were serogroup W. 97.5%, and 84.6% of the cases in Northern and Upper West regions respectively, were also serogroup W. From 2018-2020, however, except the Northern region in 2018 (74.2%), Brong Ahafo and Upper West regions in 2019 (100%) where a more significant percentage of the cases were serogroup W, the rest have more significant percentage of the cases being serogroup X (as shown in Table 5). Although, the serogroups Wand X are showing downward and upward trends, respectively, across all the reporting regions, their yearly changes are only significant (p=0.05for both serogroups W and X) in the Northern region.

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Table 6: Distribution/Trend of the various *Neisseria meningitidis* serogroups confirmed by PCR by Sex/Gender and year

Sex	Serogroups	2016 N=94 n (%)	2017 N=120 n (%)	2018 N=59 n (%)	2019 N=56 n (%)	2020 N=66 n (%)	Total N=395 n (%)	p-value
Male	B	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.00	0 (0.0)	1 (0.4)	0.48
	C	1 (1.9)	1 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.9)	0.05
	NG	0 (0.0)	0 (0.0)	4 (11.4)	2 (6.7)	1 (2.5)	7 (3.1)	0.45
	W	50 (96.2)	64 (97.0)	19 (54.3)	18 (60.0)	5 (12.5)	156 (70.0)	0.14
	X	1 (1.9)	0 (0.0)	12 (34.3)	10 (33.3)	34 (85.0)	57 (25.6)	0.14
Female	B	0 (0.0)	0 (0.0)	1 (4.2)	0 (0.0)	0 (0.0)	1 (0.6)	1.00
	NG	0 (0.0)	0 (0.0)	1 (4.2)	3 (11.5)	1 (3.8)	5 (2.9)	0.21
	W	42 (100.0)	52 (96.3)	17 (70.8)	10 (38.5)	5 (19.2)	126 (73.3)	0.01
	X	0 (0.0)	2 (3.7)	5 (20.8)	13 (50.0)	20 (76.9)	40 (23.3)	0.01

B, C, W and X- *Neisseria meningitidis* Serogroups B, C, W and X, respectively. NG-Non-groupable serogroups

4.7 Trends of Serogroups in the various Sex groups

100% of cases in females and 96.2% of cases in males were serogroup W in 2016. 97.0% and 96.3% of the cases in males and females, respectively, in 2017 were serogroup W. The same serogroup (W) was responsible for 54.3% and 70% in males and females,

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respectively, in 2018. In 2019, in the male group, 60% of the cases were serogroup W and 33.3% and 6.7%were serogroup X and NG, respectively. However, in the same year, 50% of the cases in the female group were serogroup X and 38.5% and 11.5% were serogroups W and NG, respectively. Finally, 85% and 76.9% of cases in males and females, respectively, in 2020 were serogroup X. Although there has been a downward trend of serogroup W and upward trend of the serogroup X in both males and females, the yearly changes are only significant in the female group (shown in table 6).

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CHAPTER FIVE

5.1 DISCUSSION

Cerebrospinal meningitis (CSM) due to *Neisseria meningitidis* is a contagious disease. Viesseux gave the foremost vivid account of CSM outbreak in 1806 following a classical epidemic in Geneva, Switzerland[87]. *Neisseria meningitidis* meningitis epidemic is a very serious medical emergency with public health and socioeconomic implications. *Neisseria meningitidis* was first defined in 1884[88], and isolated from cerebrospinal meningitis patients by Weichselbaum in Vienna in 1887 as a gram negative diplococci. Based on the antigenicity of its capsular polysaccharides, it was classified into thirteen serogroups[89], including A, B, C,D, H, I, K, L, W135, X,Y, Z, Z` with 90% of invasive cases caused by serogroups A, B and C. Traditionally, Y and W135 occasionally caused diseases, however, since the year 2000, outbreaks and sometimes epidemics are the results of W135[90-93]. Epidemics of meningitis due to *Neisseria meningitidis* are often difficult to predict, usually leading to delayed initiation of control measures such as immunization, resulting in poor outcome[87].

Therefore, this reports establishes a five-year trend of cerebrospinal meningitis causing *Neisseria meningitidis* serogroups in Ghana's meningitis belt. The study reports 16.3% (395/2426) of all suspected cases of cerebrospinal meningitis and as high as 44.5% (395/887) of all confirmed positive CSM cases by real-time PCR at the Tamale Public Health Laboratory within the study period to be caused by various serogroups of *Neisseria meningitidis.*

Generally, the study found out that since the outbreak of serogroup W in Ghana in 2016, cases of meningitis due *Neisseria meningitidis* had been on the downward trajectory until 2020 where there was a marginal spike in the number of cases resulting from an outbreak (16.1%, 11.0%, 8.8% and 24.4% for 2017, 2018, 2019 and 2020 respectively). The general downward trend of Neisseria meningitidis cases from 2017 to 2019 could be primarily

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explained by the 2016 massive reactive vaccination campaign with meningococcal polysaccharide ACW vaccine following the outbreak serogroup W in 2016[38]. With this, it was expected that some immunity would be achieved leading to a reduction of the yearly cases of meningitis due to *Neisseria meningitidis* serogroup W. Since serogroup W constitutes a larger percentage of cases due *Neisseria meningitidis*, you would expect that the overall cases due *Neisseria meningitidis* would decline. The increase in the number of cases in 2020 was however due to the recently reported outbreak resulting from a non-vaccine type serogroup.

The study reports significant yearly changes of both serogroups W (p=0.01) and serogroup X (p=0.01) over the five years. Although, the serogroup W, though saw a little increase in the number of cases in 2017 as compared to that in 2016, it has been on a downward trend (Std. J-T statistic= -2.45) since then, accounting for only 15.2% of total *Neisseria meningitidis* cases in the year, 2020, as against 96.7% in 2017. The serogroup X, however, has consistently been on the upward trajectory (Std. J-T statistic= 2.45) since 2016, making up 81.8% of the total *Neisseria meningitidis* cases in 2020 as against 1.1% in 2016. This finding by the current study is consistent with a recent nine-year study which was carried out in 2019 in Niger to establish the epidemiology of bacterial meningitis since the introduction of the meningococcal serogroup A conjugate vaccine. A similar trajectory of the serogroups W and X as the current study was established by that study[94].

However, the trends exhibited by these two serogroup (W and X) do not come as a surprise. There was a reactive vaccination campaign of meningococcal polysaccharide ACW vaccine in the districts affected by the 2016 *Neisseria meningitidis* serogroup W[38] outbreak, so it was expected that some population immunity would be achieved and result in reduction in the number of cases due to serogroup W *Neisseria meningitidis* as time goes by. The downward trend of the serogroup W from 2017 to 2020 is therefore, attributable to population immunity resulting from the vaccination campaign and community education on precautionary measures.

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Of note, however, is that, unlike serogroup A which was eliminated in the country just about two (2) years after the introduction of the monovalent meningococcal A conjugate vaccine, there is still the persistence of serogroup W, even four (4) years after the vaccination campaign of the polysaccharide meningococcal ACW vaccine, though the coverage rates for both campaigns were the same (98% for the monovalent meningococcal A conjugate vaccine in 2012 and over 98% for the polysaccharide meningococcal ACW vaccine in 2016)[95, 96]. This phenomenon may be explained by the relative effectiveness of conjugate and polysaccharide vaccines. Both have been demonstrated to be effective. However, most polysaccharide vaccine induce hyporesponsiveness and are less immunogenic in children under 2years and are unable to induce immunologic memory and affinity maturation in older children and adults as compared to conjugate vaccines[97, 98].

Moreover, it must be noted that, unlike the meningococcal A vaccine, which had since 2016 been incorporated into the routine vaccination program in the country, the polysaccharide meningococcal ACW is still not incorporated into the routine vaccination program. This, I believe, may have led to dilution of herd immunity resulting from birth and immigration due to the lack of an ongoing vaccination program. Hence the persistence of the serogroup W, though, a decline in numbers. The lack of continuous vaccination or its incorporation into the routine vaccination program could also lead to future epidemics resulting from the same serogroup as immunity of the sensitized or vaccinated wanes. Therefore, it is necessary that it is incorporated into the routine vaccination program to prevent future epidemics.

However, the upward trend of the serogroup X maybe attributable to the unavailability of vaccine program, coupled with highly unsensitized or susceptible population. The emergence of the serogroup X as the predominant aetiological agent in the wake of declining serogroup W in Ghana also reflects past situations wherein the wake of the decline of a previously predominant serogroup is the emergence of a different serogroup as the predominant aetiological agent. For instance, the emergence of serogroup W in 2012, when serogroup A declined after introduction of the monovalent meningococcal A

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conjugate vaccine in Burkina Faso, Mali and Niger in 2010[23-25]. Moreover, a study reports that a sizeable proportion of serogroup X during the 2017 outbreak in Togo where it constituted 37% of the cases[85]. However, unlike the recent outbreak in Ghana, which this study reports, where the serogroup X is the predominant (81.1%) agent, it was not the predominant aetiological meningococcal agent.

The serogroups B and non-groupable serogroups have largely remain stable over the study period. The significant (p=0.05) dwindling or downward trend (Std. J-T statistic= -1.94) of the serogroup C may also be explained by the vaccine availability. However, declining cases of serogroup B, even in the absence of vaccine intervention, corroborate a systematic review study which reports an overall decreasing trend, of *Neisseria meningitidis* serogroup B, particularly in countries where data collection had been consistent[99]. The number of the non-groupable serogroups has increased in 2018 and 2019 and declined slightly in the year 2020. These changes in the non-groupable serogroups across the years has however not been significant (p=0.29).

The distribution of the various serogroups in the different ages groups also follow the same trend as the cumulative cases. There is a downward trend of the serogroup W across all age groups considered in this study. Except for those under 1 year of age, where there was no reported case of serogroup X, it has been on the upward trajectory across all other age groups considered in this study. However, though there have been changes in the numbers of cases in both serogroups across all age groups across the years under study, significant changes in both serogroups were only seen in the age groups; 5-10years (p=0.05 and 0.05), 16-23years (p=0.01 and 0.01) and 24-44 years (0.04 and 0.05), for the serogroups W and X respectively. Also, consistent with a study carried out in Niger in 2019 (4.2%)[94], cases of *Neisseria meningitidis* are uncommon among children under 1year in the current study (2.0%), and all the cases in this age bracket in this study were caused be serogroup W. Except serogroup B, this study finds that cases of all the other serogroups considered in the study are most common among the age group, 5-10years. Overall, cases in this age group

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(5-10years) constitute 33% of cases of *Neisseria meningitidis* in this study. However, majority (75.8%) of cases in the Niger study, were found in the age group, 1-14yeays[94].

This study's gender distribution also follows the general trend, as described in Table 3 in the results section. However, significant changes across the years in both serogroup W and X were only seen in the female group, p=0.01 and 0.01, for serogroups W and X respectively. There is, however, no significant change in any of the serogroups in the male category.

Geographically, both serogroup W and X across all reporting regions in this study follow the same pattern as the cumulative cases. There is an increasing number of cases of serogroup X across all the regions in the wake of serogroup W decline also across all the regions. However, until 2018, the Upper West region which has recorded the highest number of cases of serogroup X in the current outbreak in Ghana, had not recorded cases of serogroup X in the recent past. Therefore, it is understandable that it experienced the outbreak since the population may have been naïve immunologically to the *Neisseria meningitidis* serogroup X. However, the Northern and the Upper East regions have consistently had cases of serogroup X since 2017. Before 2020, the Upper East region had been the region with predominant serogroup X cases. Cases of serogroup W are however, predominantly in the Northern region across all the years under study. The yearly changes of both serogroups were only significant (p= 0.05 for both serogroups) in the Northern region. However, the serogroup W changes were also significant (p=0.05) in the Upper East Region

Although there is currently no licenced vaccine targeting serogroup X available in the country and the region as whole in the wake of the rise into dominance of *Neisseria meningitidis* X in Ghana, a pentavalent meningococcal conjugate vaccine (MenACWXY) is under development for use in the African region is expected to be licenced in the year, 2021[100, 101]. It is therefore recommended that the country positions itself well by identifying sustainable funding sources so that as and when the vaccine becomes available, it is

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procured for use immediately, and in the interim, case-based surveillance and community sensitization should be intensified across all regions within the meningitis belt of the country. It is also recommended that robust surveillance is instituted for all the other non-vaccine type and the non-groupable serogroups so that an emerging threat from any is identified from the onset. Capacity for testing should also be built for all regions within the 'meningitis belt' of the country to aid rapid testing. A molecular epidemiology of the serogroup X is recommended to determine whether the strains in circulation are the same or different. This will aid the appropriate targeted approach.

5.2 Conclusion

In conclusion, the results of the analysis of the cases of bacterial meningitis due to *Neisseria meningitidis* from 2016 to 2020 shows an emergence of serogroup X, a non-vaccine type *Neisseria meningitidis* serogroup, as the predominant *Neisseria meningitidis* serogroup, and is on the upward trajectory since 2016 in the wake of a declining serogroup W after the introduction of the meningococcal polysaccharide ACW vaccine following the outbreak of serogroup W in the Upper West region in 2016. However, unlike serogroup A which has had no case recorded since 2014 after the introduction of meningococcal A conjugate vaccine in 2012, the serogroup W persists, even four (4) years after the meningococcal ACW polysaccharide vaccine mass/vaccination campaign in the country.

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